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OBSERVATIONS ON LEAF FALL IN THE DOUGLAS FIR WHEN INFECTED WITH *RHABDOCLINE PSEUDOTSUGAE* SYDOW

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OBSERVATIONS ON LEAF FALL IN THE DOUGLAS FIR WHEN INFECTED WITH *RHABDOCLINE PSEUDOTSUGAE* SYDOW

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(With Plate XLVIII.)

Introduction.

The needle-cast disease of the Douglas fir caused by the ascomycetous fungus *Rhabdocline Pseudotsugae* Syd. was first described by Weir (5) in America in 1917. In 1926 Wilson and Wilson (6) described an epidemic of the same disease in the South of Scotland, and the following account of the disease is essentially that given by those two investigators. There is reason to believe that the disease was introduced into Scotland prior to 1914, but it did not become serious until 1922.

In this country only two distinct varieties of the Douglas fir are commonly attacked, viz. the Blue Douglas, *Pseudotsuga glauca* Mayr, and an intermediate form which has provisionally been regarded as *Ps. Douglasii* var. *caesia* Schwerin. Of those two forms infection is most severe in the intermediate variety. The Green Douglas—*Ps. Douglasii* Carr.—is rarely attacked in this country, and only then to a very slight extent (1). The young leaves are infected during the summer and apothecia are developed in the following spring. After the spores have been shed, the infected leaves drop off the tree, *i.e.* when just over 12 months old. The exact mode of infection of the leaf has not been determined, but after it has taken place a mycelium of colourless septate hyphae is developed in the leaf, but does not pass back into the shoot. The cells of the infected area die, the contents become brown and there is a marked decrease in their starch content. About March the hyphae become more abundant towards the lower surface of the leaf, just below the two bands of stomata, and it is here that the apothecia are ultimately formed. The apothecia are purple brown and elongated, at first covered by the epidermis but later opening by a longitudinal slit, disclosing the orange-coloured hymenium, which consists of asci and paraphyses. The ascospores are mature by the

middle of May and infect the young leaves which have just emerged from the bud.

The object of this investigation was to establish as far as possible how and why needles infected with *R. Pseudotsugae* fall prematurely. The most obvious method of investigating the problem was to compare the abscission mechanism in healthy and infected leaves. The importance of physiological factors was, however, soon apparent, and in this respect several determinations were made of the water content of healthy leaves of different ages and of diseased leaves.

Anatomical study of the abscission mechanism in the Douglas fir.

The anatomy of leaf fall in the genus *Pseudotsuga* has already been shortly described by Neger and Fuchs (4), and the following, which is a somewhat more detailed account, confirms the work of these two investigators.

The anatomical structure of the abscission mechanism of the Blue, Green and intermediate variety of the Douglas fir was found to be exactly similar. The lamina of the needle joins the shoot by means of a short indistinctly defined petiole. Longitudinal sections, through the vascular bundle of the leaf base, including part of the shoot, reveal the following structures. At the point of junction of the petiole with the shoot and on the abaxial side, a single layer of thick-walled, pitted, lignified cells, containing simple crystals of calcium oxalate, stretches from just under the epidermis to the vascular bundle. On the adaxial side a similar layer of cells is present, but stop short some distance from the bundle (Plate XLVIII, fig. 1, *Lig.L.*). All the tissues distal to the lignified layer belong to the leaf and can be distinguished as follows. Adjacent to the thick-walled lignified cells, and having a similar distribution between the epidermis and the vascular bundle, is a band of comparatively thin-walled cells, two to three layers in thickness, both on the adaxial and the abaxial side of the petiole. These cells are small, densely protoplasmic, and each contains a large nucleus. The cell walls are permanently cellulosic and the epidermal cells dip markedly into the cuticle, which is thus much thinner here than at any other point. These thin-walled cellulosic cells just described constitute the true abscission layers (Plate XLVIII, fig. 1, *Ab.L.*). Distal to the abscission layers the cells are larger, thicker walled and elongated in the direction of the length of the leaf. The epidermal cells and three to six layers of sub-epidermal cells are strongly lignified (Plate XLVIII, fig. 1, *Hyp.L.*), constituting a rigid cylinder, the

strength of which accentuates the weakness of the abscission layers. On the abaxial side the endodermis is quite distinct and is completely lignified—in the lamina its outer tangential walls are not lignified. A lining layer of suberin can also be detected in the endodermal cells. A few cells, external to the endodermis on the abaxial side of the leaf base, also show more or less complete lignification, with not infrequent simple pits. On the adaxial side the endodermis is not well defined, the cell walls are thin and not lignified but suberised. Between the vascular bundle and the lignified epidermal and sub-epidermal cells on the adaxial side the cells are large, irregularly arranged, thin-walled, with a lining layer of suberin in many cases, and with scant protoplasmic contents.

On the proximal side of the thick-walled lignified cells at the base of the leaf, the cells are strongly suberised and are characterised by a greater or less infiltration of resin, which takes the lignin stains, and the presence within them of simple crystals of calcium oxalate. This constitutes a protective layer which is continuous with the stem periderm (Plate XLVIII, fig. 1, *St.Pd.*) and extends on all sides right up to the leaf trace. On the adaxial side, the phellogen is particularly active in the area between the vascular bundle and the point where the thick-walled lignified cells terminate (Plate XLVIII, fig. 1, *Pro.L.*). Thus, at the base of the petiole, there is a band of thin-walled permanently cellulosic cells, where abscission ultimately takes place, bounded above and below for most of its length by thick-walled lignified cells. Moreover, the vascular bundle is narrowest at the junction of needle and shoot.

In the above description no mention has been made of the age of the needles. All the tissues described are laid down very early in the development of the leaf, and can be seen in sections of needles taken quite near the apical bud. No further modification takes place as the needle gets older, except in the extent of development of the protective layer and the production of resin in the cells of this layer. In any individual branch the extent of the protective layer becomes proportionally greater as the leaves get older (Plate XLVIII, figs. 2, 3 and 4). But if the protective layers in leaves of the same age but from different branches on the tree are compared, it may be found that variation occurs as regards the actual amount of corky tissue comprising the protective layer. To take a particular example—two branches were obtained from the same Green Douglas fir. One, a 6-year-old lateral, was procured from fairly high up the tree. This branch bore no leaves over 5 years of age, while some of the 4-year-old leaves had already fallen. The other branch, a 10-year-old lateral, was taken from much lower down the tree, where the light

intensity was not so high. The diameter of this branch was much less than that of the other in internodes of the same age and the internodes were longer. The needles, however, remained on the branch for 8 years, but were beginning to fall in the seventh year. It was found that the protective layer in the 8-year-old needles from the shaded branch was not nearly so strongly developed as that of 5-year-old needles from the branch higher up the tree. There was also a corresponding variation in younger leaves from the two branches.

Examination of the abscission layers in the oldest leaves in midwinter and during the spring showed, in a few cases, that the cell walls of the permanently cellulosic layers had become distinctly swollen and that the cells themselves were separating from each other, due, no doubt, to solution of the middle lamella. But these leaves were still attached to the tree, and it seems as if the needles are capable of hanging on to the tree in this condition for some considerable time.

Rupture of the cuticle and of the vascular bundle is brought about by mechanical agencies. The cuticle, however, might rupture on contraction of the tissues due to desiccation. After the leaf has fallen the open end of the vascular bundle is exposed, while the cortical tissues are protected by the band of thick-walled lignified cells and the underlying corky tissue. Some time after leaf fall the cork cambium dips down and cuts through the vascular bundle some distance from the exposed surface (Plate XLVIII, fig. 7). In this way the inner living tissues are completely protected by an unbroken periderm. The production of this phellogen, which cuts through the bundle, may be related to the entrance of air by way of the exposed surface of the vascular bundle. The cells of the occluded part of the bundle die and become filled with resin. Nothing of the nature of tylosis or blockage of the tracheids before leaf fall has been observed.

Anatomical study of infected leaves.

The mycelium of the fungus is confined to the lamina of the needle, and in no case have hyphae been observed in the petiolar region. There is absolutely nothing of the nature of an antagonism between the fungus and the host at the point of union of leaf and shoot. This differs entirely from the state of affairs that obtains in the case of spruce (*Picea excelsa*) needles infected with *Lophodernum macrosporum*. Examination of a limited amount of preserved material of diseased *P. excelsa* showed that, in this case, there is undoubtedly an antagonism between the fungus and the host at the point of union of the leaf with the leaf cushion. Just at this point there is a single layer of thick-walled cells stretching from the

epidermis right up to the vascular bundle on all sides. These cells are markedly suberised and appear to form an impassible barrier to the fungus, which is thus confined to the leaf. The cells immediately distal to this suberised layer are obviously modified by the presence of the fungus in so far as the cell walls take on a very dark colour which is visible to the naked eye as a black ring round the base of the leaf, where it joins the leaf cushion. These observations confirm the work of Neger(3) on this subject.

In the lamina of the infected Douglas fir needle the endodermis is particularly heavily infected by the fungus on the abaxial side, but hyphae have not been observed within the endodermis. Infected leaves are somewhat thinner than healthy leaves, due, no doubt, to the destruction of the protoplasm of the leaf cells, with consequent loss of turgidity. Sections were cut to see if the fungus had any effect on the thickness of the cuticle, but no difference between healthy and infected leaves of the same age could be detected.

The appearance and staining reactions of the tissues of the leaf base were exactly similar in infected and healthy leaves (Plate XLVIII, figs. 5 and 6), but infected needles fall prematurely when just over a year old. The limited extent of the fungus in the leaf and the absence of any anatomical modifications of the tissues are convincing evidence of the importance of physiological factors at work in bringing about the ultimate pathological condition of the Douglas fir host. The structure of the leaf scar is in no way different, after the needles have fallen from the normal scar, except that the corky protective layer is not so strongly developed.

The possibility of the production of toxins by the fungus, as the causal agent in defoliation, has been considered, but in the opinion of the writer this is improbable, firstly, because in the diseased leaf definite areas of infection can often be recognised between which the cells of the leaf appear to be quite normal; and secondly, because in the true abscission layers themselves the cells are in no way different from those of healthy leaves.

Water content experiments[1].

The average weight of leaves used in these determinations was about 10 gm., and in any single experiment the leaves were taken from the same lateral branch of the main shoot. The water content was determined by

[1] For these experiments the author was unable to obtain healthy material of the intermediate variety of the Douglas fir—*Ps. Douglasii* var. *caesia*—but the results show that a comparison of the water content of leaves of the same age from the Green, Blue and intermediate varieties is quite justified.

heating a known weight of leaves in an electric oven, at a constant temperature of 105° C., until successive weighings showed that all the water in the leaves had been driven off. The results of these experiments are shown in Tables I and II.

In the healthy tree (Table I) there is, on the whole, a gradual decrease in the water content of the leaves as they get older. The water content of the 1-year-old leaves is always the greatest.

Table I.

Water content of leaves from healthy trees.

Species	Location	Date of collection	Water content (%) 1 year	2 years	3 years	4 years	5 years	6 years
Pseudotsuga Douglasii	R.B.G.*	29. x. 28	62	60·88	59·06	57·94	—	—
„	„	14. xi. 28	61·89	60·61	56·22	59·96	59·88	57·78
„	„	6. xii. 28	64·14	63·54	61·82	61·05	60·37	58·97
Pseudotsuga glauca	Glentress Peebles	20. xi. 28	59·64	58·79	56·69	55·76	56·41	56·97
„	R.B.G.	15. i. 29	59·79	56·84	54·78	55·62	53·85	53·63

* R.B.G. = Royal Botanic Garden, Edinburgh.

In the diseased tree it is the 1-year-old or current year's leaves that are infected and, as is shown in the first part of Table II, which presents the results obtained before the apothecia had opened, the water content of the infected leaves is distinctly lower than that of healthy leaves of the same age, whereas the water content of the older non-infected leaves from diseased trees is similar to that of leaves of the same age from healthy trees. Moreover, in the diseased trees the water content of the infected leaves is lower than that of any other leaves present on the tree.

As has been already stated, there is no obvious difference between the cuticle of healthy and diseased leaves of the same age, but sections of the infected needles used in the experiments recorded in Table II showed that the mycelium had begun to aggregate under the two bands of stomata on the underside of the leaf in preparation for the formation of apothecia, and in some instances hyphae were observed within the stomatal cavity and protruding almost to the exterior. Hyphae have not been observed in the guard cells. It may be that, after infection has proceeded so far, the stomata are no longer functional and are unable to control transpiration. Moreover, the presence of abundant hyphae in the sub-stomatal cavity greatly increases the surface area of that region, so that excessive transpiration is quite understandable.

Table II.

Water content of leaves from diseased trees before and after the fructifications have opened. (The 1-year-old needles only are infected*.)

Species	Location	Date of collection	Water content (%) 1 year	2 years	3 and 4 years	5 and 6 years	Remarks
Ps. Douglasii var. *caesia*	Glentress Peebles	4. ii. 29	52·70	Defoliated	58·32	57·24	Fructifications closed
„	„	„	52·24	„	55·12	56·65	
„	„	„	53·31	„	56·02	55·46	
„	„	„	52·74	„	54·62	55·57	
„	„	29. iii. 29	50·53	„	58·31	58·81	
„	„	„	53·14	„	56·32	56·14	
„	„	„	50·92	„	55·74	54·66	
Ps. Douglasii var. *caesia*	Glentress Peebles	24. v. 29	50·56	„	56·42	58·52	Fructifications open
„	„	„	53·33	„	57·32	59·14	
„	„	„	46·01	„	56·13	56·02	

* The material used in these experiments bore practically no 2-year-old leaves, due to the fact that the trees had been heavily infected the previous year with subsequent more or less complete defoliation of the infected growth.

Three water content determinations were made after the apothecia had burst through the epidermis, the results of which are shown in the second part of Table II. In only one case is there any appreciable drop in the water content of the infected leaves, otherwise the results are similar to those obtained before the apothecia had reached maturity.

It is interesting here to note the results of Galloway's(2) work on the leaf-cast disease of *Pinus virginiana* due to the rust fungus *Gallowaya pini* Arth. He found that before the fungus ruptured the tissues the diseased areas lost less water than the healthy portion of the leaf, due to the permanent closing of the stomata over the diseased areas. As soon as the fungus ruptured the tissues, however, evaporation increased about one-fifth above normal. In consequence of this the reserve water in the cells was gradually used up, with a subsequent loss of turgidity of the tissues. This was followed by other physiological changes which led to the gradual death and casting of the leaves. In the case of *R. Pseudotsugae*, however, the fungus does not cause the stomata to close permanently. In fact, for some time before the fungus brings about a rupture of the tissues, the mycelium is densely aggregated in the substomatal cavities, and in sections can often be seen protruding outside the stomata altogether. This suggests that the stomata are permanently open, a fact which would help to explain the marked drop in the water

content of diseased leaves, prior to the rupture of the tissues, and the absence of a further drop when the apothecia are mature.

A consideration of Table II reveals just a suggestion that the water content of healthy leaves nearest the infected areas may be affected by the presence of the disease, for in five out of the ten determinations made the water content of the 5- and 6-year-old leaves is greater than that of the 3- and 4-year-old leaves by amounts varying between 0·5 and 2·1 per cent. Many more determinations would need to be made, however, before anything conclusive could be available in this respect.

Discussion of results.

In investigating a problem such as this, there is a tendency to draw one's conclusions as to the factors at work in the abnormal, *i.e.* the diseased, host from one's knowledge of the normal. But fundamentally there is no logical reason to suppose that processes in the abnormal should not reveal, or at least give a clue to those acting in the normal host.

This study has shown that the abscission layers and associated tissues are laid down very early in the development of the leaf. No further modification takes place, except in the extent of development of the protective layer which increases proportionally as the leaves get older. It has also shown that the protective layer varies in leaves of the same age from the same tree, and that the development of the protective layer just before leaf fall is by no means constant. Hence it would appear that the extent of development of the protective layer is dependent on external growth conditions and has no causal relationship with leaf fall.

In nature the leaves do not fall in strict progression in respect to age, which suggests that leaf fall is not due to the completion of an abscission mechanism from the histological point of view. This idea is further strengthened by the fact that, in the diseased host, the infected leaves fall when just over a year old, with no abnormality of the abscission layers. Moreover, the complete defoliation of infected leaves is adequate proof that the abscission mechanism is efficient at an early stage in development of the leaf under certain physiological conditions, and this idea can probably be extended to the normal host also.

To say that the defoliation of infected leaves of the Douglas fir is ultimately due to physiological factors is practically a confession of ignorance of the causal factors involved, and the author does not pretend to think that any satisfactory explanation on physiological lines is forthcoming from this investigation. All he is prepared to say is, that the presence of *R. Pseudotsugae* brings about a marked decrease in the water

content of infected leaves, which decrease is probably of a causal nature in the ultimate pathological condition of the Douglas fir host. That there is a correlation between the water content of the leaves and leaf fall seems obvious from a consideration of Tables I and II. It has been shown (Table I) that, in the case of the healthy Douglas fir, as the leaves get older there is a gradual decrease in the water content. The possibility that this decrease is an instrumental factor in leaf fall is, if anything, strengthened by the fact that there is no strict successional defoliation in respect to age, and also by the fact that the abscission layer and associated tissues are completed at a very early stage in the development of the leaf. Also, it has been shown (Table II) that infection with *R. Pseudotsugae* brings about a marked decrease in the water content of affected leaves. Bearing in mind that it is only 5-year-old leaves that are infected, that the abscission layers and associated tissues are not invaded by the fungus, and the subsequent complete defoliation of infected leaves, it seems impossible not to admit of the intimate relationship between water content of the leaves and leaf fall.

Summary.

1. In the Douglas fir the abscission layers and associated tissues are laid down early in the development of the leaf.

2. The extent of development of the protective layer has no causal relationship with leaf fall.

3. Infection with *R. Pseudotsugae* brings about a marked decrease in the water content of infected leaves.

4. The abscission mechanism is efficient at an early stage in the life of the leaf, provided the necessary physiological conditions are present and *R. Pseudotsugae* is capable of providing these conditions.

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EXPLANATION OF PLATE XLVIII

Fig. 1. Diagram of longitudinal section of leaf base of the Douglas fir. × 75. *Lig.L.* = Thick walled pitted lignified cells at point of union of leaf and shoot. *Ab.L.* = Permanently cellulosic layers where abscission takes place. *Hyp.L.* = Ridge of epidermal and hypodermal lignified cells. *Pro.L.* = Protective layer at point where the phellogen is most active—on the adaxial side of the vascular bundle. *St.Pd.* = Stem periderm. *End.* = Endodermis. *V.B.* = Vascular bundle.

Fig. 2. Diagram of longitudinal section of a 1-year-old leaf base of the Douglas fir. × 60.

Fig. 3. Diagram of longitudinal section of a 6-year-old leaf base of the Douglas fir. × 60.

Fig. 4. Diagram of longitudinal section of an 8-year-old leaf base of the Douglas fir. × 60.

Figs. 2, 3 and 4 were drawn from material obtained from the same branch. They show the development of the protective layer (*Pro.L.*) as the leaves get older.

Fig. 5. *Ps. Douglasii* var. *caesia*—microphotograph—longitudinal section of a 1-year-old, disease free, leaf base. × 70. Lettering as in Fig. 1.

Fig. 6. *Ps. Douglasii* var. *caesia*—microphotograph—longitudinal section of a 1-year-old leaf base infected with *R. Pseudotsugae*. × 70. Lettering as in Fig. 1.

Fig. 7. Microphotograph, longitudinal section of a leaf scar of the Douglas fir in a 5-year-old internode. × 70. *V.B.* = Occluded part of vascular bundle. *Ph.* = Phellogen.

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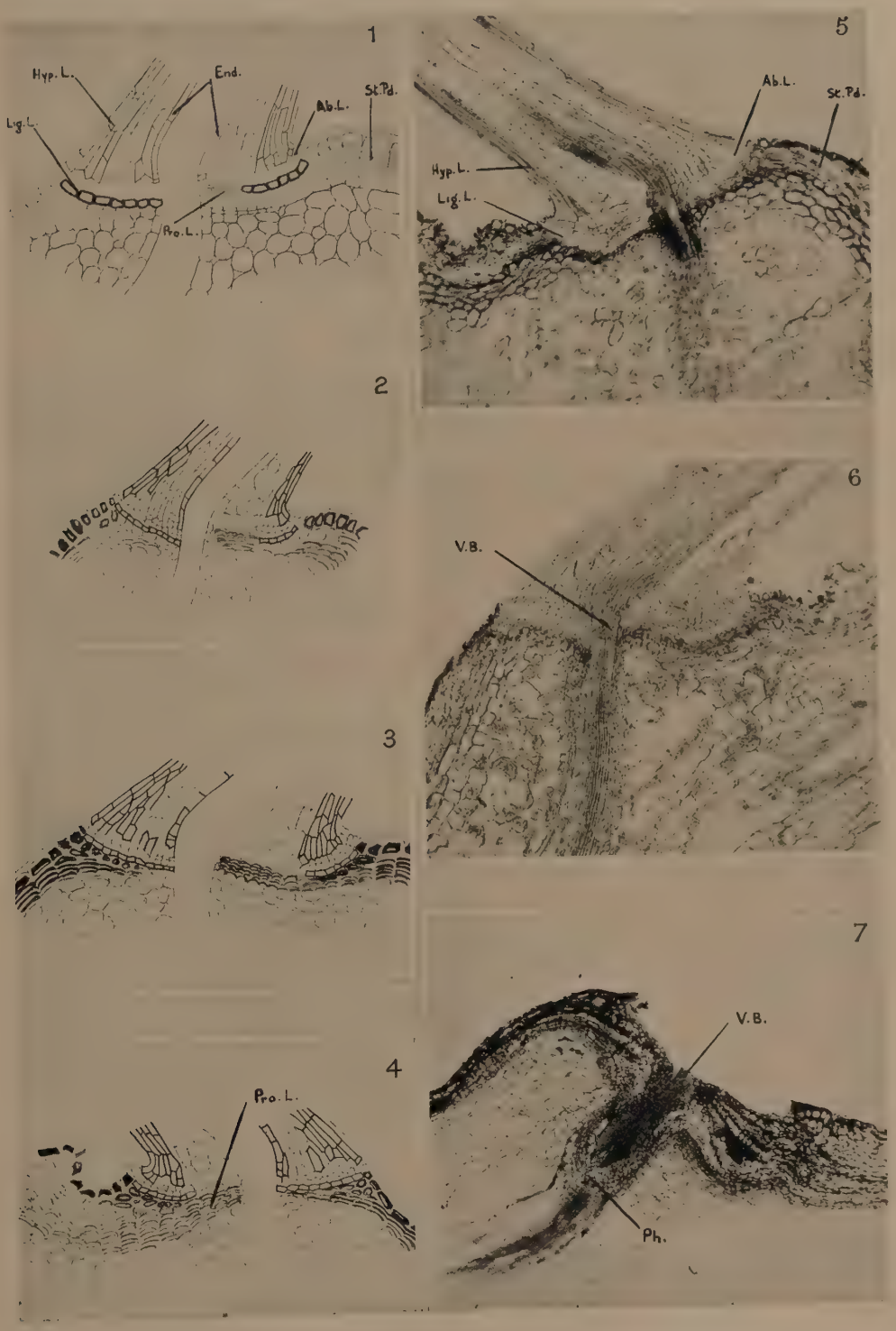

BROWN.—Observations on Leaf Fall in the Douglas Fir when infected with *Rhabdocline Pseudotsugae* Sydow (pp. 745–754).